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14. ABSTRACT
The goals attained durring this project are: (1) Development of extremely computational methods to study allosteric dynamis in enzymes, molecular motos, and chaperones. (2) Application of these techniques to closed to open transition in the enzyme DHFR. The results were in excellent with experiments. More importantly, our predictions were quantitatively validated in NMR experiments. (2) A fully quantitative simulation of the folding landscape of riboswitches. Remarkably, our predictions were verified in 2011 in single molecule experiments fully three years after the predictions. (3) Identified residues that are responsible for transmitting allosteric signals in motors and chaperones.

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1. REPORT DATE. Full publication date, including day, month, if available. Must cite at lest the year and be Year 2000 compliant, e.g., 30-06-1998; xx-08-1998; xx-xx-1998.

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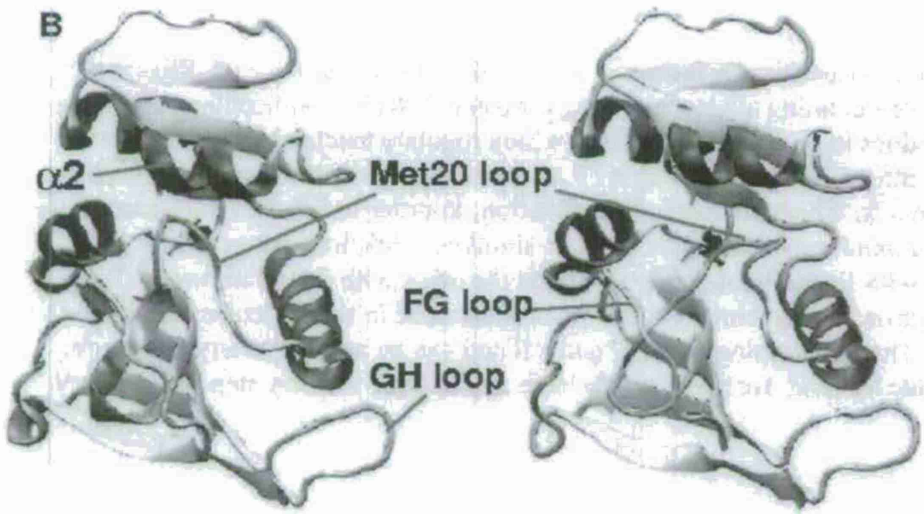
FINAL REPORT (FA9550-07-1-0098) PI: D. Thirumalai, University of Maryland

The funding was used to develop computational methods to understand the folding of proteins and functions of molecular motors. We made substantial progress in both fronts. The highlights of the grant are: (1) Development of theoretical and computational methods to understand allosteric transitions in enzymes and motors. (2) Application of these ideas to understand, at the molecular level, transitions from one state to another. Specific applications include transitions in DHFR, myosin, and bacterial chaperonin GroEL. (3) We have also proposed ways of understanding and interpreting experiments on riboswitches, genetic sensors. A few highlights follow.

Allosteric Wiring Diagram: We developed a direct sequence-based method for obtaining allosteric wiring diagram in enzymes and molecular machines. The function and dynamics of machines is encoded in the structure. Upon function communication across the residues that are dispersed throughout the structure occurs. The key question is what is the signaling pathway that controls this communication? In other words from structures can one predict the allosteric wiring diagram (AWD)? More importantly, are there evolutionary imprints that preserve the nature of the wiring diagram?

To answer this important question we developed both a sequence-based method, which exploits evolutionary signals, to predict the residues that communicate the signals for functional purposes. We applied the computational method to DHFR (see the two distinct structures in the CS and OS states in the diagram below). The sequence-based method showed, surprisingly, that a very sparse network of residues that are interspersed throughout the structure, determines AWD. To further test their role we developed a dynamical simulation model that monitors the kinetics of the CS to OS transitions. Remarkably, the sliding of residues along the helices (purple in the diagrams on the right) that appear stationary from crystal structures plays a crucial in the CS to OS transition. DHFR controls cell growth and has been a cancer target and has been studied extensively. Nevertheless, this work shows for the first time a detailed "movie" of motions of sparse network of residues in the AWD that control dynamics.



Riboswtich Landscape: Riboswitches found in the untranslated regions of mRNAs of both prokaryotes and eukaryotes, are RNA elements that regulate gene expression by sensing and binding target cellular metabolites. They contain a conserved metabolite-binding aptamer domain and a downstream expression platform. In bacteria, ligand binding to the aptamer domain usually results in a conformational change,2 which alters the folding pattern of the expression platform and controls transcription termination or translation initiation. Among the simplest riboswitches, the purine (guanine and adenine) riboswitches display remarkable ligand selectivity and carry out markedly different functions despite the structural similarity of their aptamers. For the *pbuE* adenine (A) riboswitch, the ligand binding activates the gene expression when an antiterminator is formed.5 In the absence of adenine, part of the aptamer region is involved in the formation of a terminator stem with the expression platform, which results in transcription termination. The *add* A-riboswitch, on the other hand, activates the gene expression by forming translational activator upon ligand binding, while, in the absence of adenine, the riboswitch adopts the structure with a translational repressor stem in the downstream region. Thus, it is important to quantitatively map the folding landscape of aptamers to understand the differences in the function of structurally similar riboswitches.

We developed a method for the force(*f*)-triggered unfolding and refolding of the A-riboswitch aptamer theoretically using the self-organized polymer model8 with the Langevin dynamics in the overdamped limit. The native structure, taken from the crystal structure of the aptamer domain of the *Vibrio* V*ulnificus add* A-riboswitch has 63 nucleotides. Our results yielded quantitative support for experiments, and provided for the first time the entire landscape of riboswitches that could be compared with experiments. The result is crucial for transcription of the complete riboswitch. In vivo, without metabolite binding, the riboswitch favors the formation of the downstream terminator hairpin, which disrupts the aptamer structure. Ligand binding thus stabilizes the aptamer structure during transcription and prevents the formation of the terminator stem before transcription is completed (*pbuE* riboswitches) or the formation of translation repressor stem before translation is initiated (*add* riboswitches). Further study of cotranscriptional folding of the complete riboswitch including the downstream expression platform is necessary to fully understand the mechanism of gene regulation by riboswitches.

Conformational transitions in motors: We had developed and tested a direct structure-based method for obtaining allosteric wiring diagram (AWD) in molecular. AWD is a network of residues in biological machines, which regulate mechanical movements in response to binding of ligands such as ATP. As such they carry the signals for such domain movements, which are needed for function. In order to link this to dynamics we developed a computational technique that can simulate transitions between any two states, say one with ligand bound (apo state)and the other with ligand unbound (holo state). The function and dynamics of machines is encoded in the structure, and linked to the dynamics. The methodology is very general and can be applied to any system for which large scale motions are sought. We have applied this to a key step in myosin V transition.

Myosins are a family of motors that move along actin filaments to transport cargo in cells. To elucidate the structural changes that take place in the rigor (R) to post-rigor (PR) transition in MyosinV, which result in the detachment from actin, we used a combination of an elastic network model and Brownian dynamics using the SOP energy function. We showed that the allostery wiring diagram is made up of a network of residues that connect the ATP and actin binding regions.Several of the residues in the AWD have been shown to be important in the allosteric transitions associated with myoV.

Remarkably, the structural elements associated with the AWD are found to be responsible for driving the kinetics of the R $\rightarrow$ PR transition. The dynamical simulations show that the exponential kinetics associated with the global dynamics masks the hidden complexity of the movements associated with the key structural elements in the R $\rightarrow$ PR transition. The hierarchy of time scales that drive the global conformational change of the motor domains begins with the movement of switch I towards the P-loop (Fig. 1). The two structural elements move towards their post-rigor positions on a timescale of which is a factor of two less than that associated with the decay of the global motion. The coordinated movement of P-loop and switch I triggers a concerted rearrangement of the rest of the structure, in particular the rotation of H18 and the entire U50 domain with respect to the L50 domain. It is the relative shift of U50, carried by H18, and L50 that opens the cleft between the two domains and causes the myosin motor domain to disassociate from actin. Thus, the entire domain has to move in a hierarchical manner to affect the global transition, and hence facilitate the movement of the motor on actin. Two papers are currently being prepared for publication.